Clinica Chimica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Novel LC–MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact

Matthijs Oyaert [a,*], Nele Peersman [a], Davy Kieffer [a,b], Kathleen Deiteren [c], Anne Smits [d,e], Karel Allegaert [d,e], Isabel Spriet [f,g], Johan Van Eldere [a,b], Jan Verhaegen [a,b], Pieter Vermeersch [a,h], Steven Pauwels [a,h]

[a] University Hospitals Leuven, Department of Laboratory Medicine, B-3000 Leuven, Belgium
[b] KU Leuven — University of Leuven, Department of Microbiology and Immunology, B-3000 Leuven, Belgium
[c] Antwerp University Hospital, Department of Laboratory Medicine, Edegem, Belgium
[d] KU Leuven — University of Leuven, Department of Development and Regeneration, B-3000 Leuven, Belgium
[e] University Hospitals Leuven, Neonatal Intensive Care Unit, B-3000 Leuven, Belgium
[f] KU Leuven — University of Leuven, Department of Clinical Pharmacology and Pharmacotherapy, B-3000 Leuven, Belgium
[g] University Hospitals Leuven, Pharmacy Department, B-3000 Leuven, Belgium
[h] KU Leuven — University of Leuven, Department of Cardiovascular Sciences, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 14 August 2014
Received in revised form 8 December 2014
Accepted 10 December 2014
Available online xxxx

Keywords:
LC–MS/MS
Vancomycin
Tandem mass spectrometry
Therapeutic drug monitoring

ABSTRACT

Background: Accurate quantification of vancomycin in plasma is important for adequate dose-adjustment. As literature suggests between-method differences, our first objective was to develop a novel liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for total vancomycin in human plasma and to compare frequently used immunoassays with this method. Secondly, we investigated the clinical impact of between-method quantification differences.
Methods: For LC–MS/MS, lithium heparin plasma was extracted by adding a precipitation reagent containing the internal standard (vancomycin-des-leucine). Analysis was performed on an Acquity TQD mass spectrometer equipped with an Acquity UPLC 2795 separations module. Our method was analytically validated and compared with four frequently used immunoassays from four different manufacturers. Vancomycin concentrations were clinically classified as toxic, therapeutic and sub-therapeutic. Clinical discordance was calculated using LC–MS/MS as a reference.
Results: A novel LC–MS/MS method using protein precipitation as sole pretreatment and an analysis time of 5.0 min was developed. The assay had a total imprecision of 2.6–8.5%, a limit of quantification of 0.3 mg/L and an accuracy ranging from 101.4 to 111.2%. Using LC–MS/MS as reference, three immunoassays showed a mean proportional difference within 10% and one showed a substantial mean proportional difference of >20%. Clinical discordant interpretation of the obtained concentrations ranged from 6.1 to 22.2%.
Conclusions: We developed a novel LC–MS/MS method for rapid analysis of total vancomycin concentrations in human plasma. Correlation of the method with immunoassays showed a mean proportional difference >20% for one of the assays, causing discordant clinical interpretation in more than 1 out of 5 samples.

1. Introduction

Vancomycin is a glycopeptide antibiotic with strong bactericidal activity against gram-positive bacteria. These do not only include methicillin-resistant *Staphylococci*, but also penicillin resistant organisms, such as *Streptococci* and *Corynebacteria* [1]. Large inter- and intra-patient variability, combined with a correlation between low plasma concentrations and therapeutic failure on the one hand, and high plasma concentrations and toxicity on the other hand, makes the molecule an excellent candidate for therapeutic drug monitoring (TDM). In addition, the potential rise in minimum inhibitory concentrations of vancomycin target organisms makes it increasingly important to adjust its dosage in order to ensure adequate concentrations [2,3]. In clinical practice, therapeutic intervals, target levels and dose-adjustment schemes in function of administration mode and sampling time are used.

Current recommendations, however, do not take into account that routine plasma vancomycin quantification by commercial immunoassays can show substantial between-method differences [4–6]. Next to standardization issues, immunoassays can also lack specificity. For

Abbreviations: LC–MS/MS, liquid chromatography–tandem mass spectrometry; TDM, therapeutic drug monitoring; IS, internal standard; QC, quality control; MRM, multiple reaction monitoring; S/N, signal to noise ratio; CV, coefficient of variation; ME, matrix effect; DR, detection range; FPIA, fluoro polarized immuno assay; PETINIA, particle enhanced turbidimetric inhibition immunoassay; LOQ, limit of quantification; LOA, limit of agreement

* Corresponding author at: University Hospitals Leuven, Laboratory Medicine, Herestraat 49, B-3000 Leuven, Belgium. Tel.: +32 16 34 70 00; fax: +32 16 34 79 31.
E-mail address: matthijsoyaert@telenet.be (M. Oyaert).

http://dx.doi.org/10.1016/j.cca.2014.12.012
0009-8981/

example, cross-reacting substances such as vancomycin degradation products have been described to interfere with some immunoassays [7]. Also, several structurally related compounds are formed during the production process and can be present in the isolated substance. A study of Diana et al. investigated the impurities present in a commercial vancomycin sample and found 15 different impurities, together composing 16.6% of the sample [7]. The *clinical impact* of these issues was recently suggested in a paper by Zhao et al., in which the predictive performances of different neonatal pharmacokinetic models for vancomycin administration were compared [8]. They found different predictive performances between different analytical methods for serum vancomycin concentrations, thereby highlighting that dosage individualization of vancomycin in neonates should not only consider patients' characteristics like body weight, but also the methods used to measure vancomycin [9]. Moreover, it remains often difficult to track the analytical details of the methods used to measure vancomycin in determining therapeutic intervals and target values [10], thereby shedding doubt on the applicability of the guidelines in specific hospital settings. Lastly, current guidelines use the total concentration of vancomycin (free and bound) for dosage adjustment [11], even though it is known that, as for most antibiotics [12], it is probably the free concentration that is critical for diffusion into infected areas [13,14]. Whether reported protein-binding percentages for vancomycin are stable and predictable when only looking at total vancomycin concentrations is part of an ongoing discussion. The same holds true for the added value of measuring free concentrations.

To tackle the above-mentioned limitations, a number of methods using mass spectrometry (LC–MS/MS) for the quantification of plasma total vancomycin concentration have been described. These methods, however, rely on internal standard (IS) compounds that are structurally not related to the target analyte (teicoplanin, atenolol, kanamycin-B) [15–18], use a labor-intensive sample preparation [5,16], or have very long runtimes [5].

It is known that the IS has a crucial role in compensating for sample specific matrix effects (MEs) in LC–MS/MS assays. As most studies relied on other, structurally and hence physically and chemically unrelated compounds as IS, it is not surprising that significantly different percentages of ME between vancomycin and IS (up to 50% difference [18]) have been described in published methods, shedding serious doubt on the quantification accuracy of clinical samples presenting with varying matrices. A recently published method tried to cope with this problem by synthesizing a homemade vancomycin derivative [5]. Although this method was intended as a reference method, the use of a homemade vancomycin derivative as IS is time-consuming and offers no workable solution for other groups trying to easily measure vancomyin with mass spectrometry. Moreover, this method has an analysis time of up to 20 min per sample [5].

The first aim of our study was to develop a novel LC–MS/MS method for measuring total vancomycin concentrations with acceptable runtimes and using an adequate IS. To chart between-method differences, we compared four frequently used immunoassays with our method. In addition, we investigated the clinical impact of the observed differences.

2. Materials and methods

2.1. Chemicals and solutions

Vancomycin HCl was purchased from Toronto Research Chemicals (Toronto, Canada). Vancomycin-des-leucine formiate was purchased from Alsachim (Strasbourg, France; formulation on request) and acetonitrile (LC–MS grade) from BioSolve (Valkenswaard, The Netherlands). HPLC-grade water was generated using a Milli-Q-water-purification system (Millipore, Molsheim, France). Pooled blank lithium heparin (Becton Dickinson, Franklin Lakes, USA) blood samples were collected from a healthy volunteer.

A stock solution of vancomyin in water at 4.0 mg/mL was prepared. Ten calibration standards at vancomycin concentrations of 0.6, 1.3, 2.5, 5.0, 10.0, 20.0, 30.0, 50.0, 75.0 and 100.0 mg/L were prepared by appropriate addition of stock solution to the blank plasma pool. An IS working solution of 5.0 mg/L vancomycin des-leucine in Milli-Q was used. In each routine analysis, four levels of quality control (QC) (3.0, 15.0, 30.0 and 75.0 mg/L) were analyzed. These were prepared by the appropriate addition of another (independently prepared and weighed) stock of vancomycin (4.0 mg/mL in water) to blank pooled plasma. QCs, calibration standards and IS working solution were stored at −20 °C until use.

2.2. Sample preparation and LC–MS/MS conditions

Lithium heparin blood samples were centrifuged for 10 min at 1912 *g*. 40 μL plasma was immediately vortexed with 40 μL IS working solution and 160 μL acetonitrile in glass tubes. After centrifugation (10 min at 16,100 *g*), 5 μL of supernatant was injected (auto-sampler) into the chromatographic system. Chromatographic separation was carried out on an Acquity UPLC separations module (Waters Ltd, Watford, UK). As analytical column, an Acquity UPLC BEH HILIC (100 mm × 2.1 mm; 1.7 μm, Waters Ltd, Watford, UK), maintained at 50 °C, was used with a Phenomenex C-18 guard column (100 mm × 4 mm, Torrance, CA, USA) as pre-column.

The mobile phase was a mixture of acetonitrile (buffer A) and water (buffer B) both containing 0.1% formic acid. A linear gradient starting from 95% buffer A descending to 40% buffer A at 2.50 min was applied. At 2.60 min, buffer A was set at 99% and kept till 4.00 min. From 4.00 to 5.00 min 95% buffer A was used to re-equilibrate for the next injection. The flow rate was set at 0.45 mL/min, the total runtime was 5.0 min. Mass spectrometric analysis was performed using a tandem mass spectrometer (Acquity TQD detector, Waters Ltd, Watford, UK) equipped with an electrospray ionization source operating in the electrospray-positive mode. The source and desolvation temperature were set at 150 °C and 500 °C, respectively. Nitrogen was used as desolvation gas and was set at a flow rate of 750 L/h. Capillary voltage was set at 3.5 kV, cone voltage at 20 V and collision energy at 20 eV. Vancomycin was detected by multiple reaction monitoring (MRM) with a dwell time of 0.085 s. The following MRM transitions were monitored: m/z 725.2 → 144.0 and 726.1 → 144.0 for vancomycin, and 662.1 → 144.0 for vancomycin des-leucine. Vancomycin was quantified by means of calibration to each run, using a weighted least square ($1/X^2$) regression in MassLynx software (Waters Ltd, Watford, UK) of the 10 calibration standards. For vancomycin the 2 MRMs were summed.

2.3. Analytical validation

Method imprecision was evaluated by analysis of four QC concentrations and three concentrations of patient samples on ten consecutive days [19]. A total imprecision of <15% was acceptable [20].

The limit of quantification (LOQ) was defined as the lowest analyte concentration with a signal to noise ratio (S/N) of more than 10, a coefficient of variation (CV) and accuracy ≤20% [21].

Linearity was evaluated by comparing if higher order equations give significantly better fits using Microsoft Excel Analyse-it software. To determine the amount of carry-over, we analyzed in the sequence HHHBBB, where H is the highest calibration standard and B is a blank. The percentage of carry-over was calculated with the formula 100 × (B1 − B3) / (H3 − B3) [19]. Q5

Accuracy was calculated from the QC samples (n = 4) in ten different runs as the percentage deviation from the theoretically added vancomycin concentration. An accuracy of <15% was accepted [20]. To the best of our knowledge, no reference plasma exists for vancomycin.

Freeze and thaw, short-term, and long-term stability of plasma samples were determined at three concentration levels. Freeze and thaw stability was tested by comparing freshly prepared samples to samples

Please cite this article as: Oyaert M, et al, Novel LC–MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact, Clin Chim Acta (2014), http://dx.doi.org/10.1016/j.cca.2014.12.012

that underwent six freeze–thaw cycles. Short and long-term stability was assessed by storing samples at 20 °C, 8 °C and −20 °C. The obtained results were compared with the results found in the initial analytical run. For each quantification, a fresh calibration curve was used. Extract stability was determined by measuring three vancomycin concentrations (5, 20 and 50 mg/L) 24 and 48 h after preparation, stored in the autosampler (2–8 °C). A deviation of ±20% compared to the results obtained in the initial run was accepted.

Extraction recovery was evaluated by comparing the peak areas of vancomycin, spiked in blank plasma, before and after extraction for three concentration levels (5, 20 and 40 mg/L). We evaluated ME by comparing the peak areas of vancomycin spiked at 5 and 20 mg/L in pure solvent (water), with the peak areas of vancomycin spiked at 5 and 20 mg/L in six different blank plasma extracts (three patients who received a multitude of medication, but not vancomycin; two samples of healthy volunteers and one plasma filtrate). The sample ME was calculated with the equation ME% = B/A * 100, where B refers to the peak area of vancomycin obtained in matrix and A to the peak area in solvent.

Selectivity was tested by running four samples from critically ill and hemato-oncology patients not receiving vancomycin, but a number of other frequently used medications (in total 39 other medications). The resulting chromatograms were examined for interfering peaks at the retention time of vancomycin.

2.4. Method comparison

Leftovers from lithium-heparin patient blood samples sent to the Clinical Laboratory of University Hospitals Leuven for clinically indicated vancomycin plasma measurements were used for method comparison (99 samples including 68 different patients and one external QC sample). Our study was performed with full respect for individuals' rights to confidentiality and in accordance with procedures supervised by the local authorities responsible for ethical research. Five aliquots of these samples were prepared and stored at −20 °C until analysis.

Total vancomycin plasma concentrations were measured with our LC–MS/MS assay and with four different immunoassay reagent systems. The vancomycin assay on Architect i2000SR (Abbott, North Chicago, Illinois, USA; detection range (DR): 3.0 to 100.0 mg/L, n = 98 samples analyzed) is an immunoassay based on chemiluminescence using acridinium-conjugated antibodies (Fluoro Polarized Immuno Assay (FPIA)). The vancomycin assay on the Roche Cobas 8000 c702 (Roche Diagnostics, Mannheim, Germany; DR: 1.7 to 80.0 mg/L; n = 99 samples analyzed) and Ortho Vitros 5000 (Ortho Clinical Diagnostics, Mongkok, Hong Kong; DR: 5.0 to 100.0 mg/L; n = 99 samples analyzed) are based on the competition between the drug in a sample and the drug labeled with the enzyme glucose-6-phosphate-dehydrogenase, which is included in the assay for antibody binding sites. The Siemens Dimension Vista 1500 assay (Siemens Healthcare Diagnostics, Deerfield, IL; DR: 0.8–50.0 mg/L; n = 98 samples analyzed) is a homogeneous particle enhanced turbidimetric inhibition immunoassay (PETINIA) that incorporates a monoclonal detection antibody. Intra- and inter-run coefficients of variation (CV) were evaluated by paired analysis of at least ten different runs. The repeatability (1.6 to 3.0%, 1.5 to 3.3%, 2.7 to 3.1%; and 1.5 to 3.4%) and total imprecision assay CVs (3.1 to 6.2%; 2.4 to 4.4%; 5.1 to 6.1%; and 2.7 to 3.9%) on respectively Abbott Architect i2000SR, Roche Cobas 8000, Ortho Vitros 5000 and Siemens Dimension Vista 1500, performed on internal quality control material, were satisfactory according to the respective leaflets [23–26].

To study the clinical impact of the vancomycin results obtained with the five different assays, we used the therapeutic range for vancomycin administered by continuous infusion (15.0–25.0 mg/L) [10,11] and classified the results as therapeutic (15.0–25.0 mg/L), toxic (>25.0 mg/L) or sub-therapeutic (<15.0 mg/L).

2.5. Statistics

Bland–Altman analysis, Passing Bablok regression analysis and Pearson's correlation coefficients were used to study interchangeability between results from the different immunoassays with LC–MS/MS as a reference [27,28]. Data-analysis was performed by using Microsoft Excel Analyse-it version 2.21 (Analyse-it Software Ltd, Leeds, UK).

3. Results

3.1. Analytical validation

Under the applied chromatographic conditions, vancomycin eluted as a peak at a retention time of 2.7 min. Chromatograms of a low and high QC and a patient sample (14.3 mg/L) are shown in Fig. 1. Repeatability and total imprecision were found to be <9%, (Table 1). The LOQ was 0.3 mg/L; the CV at this concentration was only 8.8%. The assay was linear in from 0.3 to 100.0 mg/L. No significant carry-over was detected (<0.5%).

The accuracy ranged from 101.4 to 111.2% for the four QC concentrations and was therefore within preset limits (<15.0%). Samples showed no substantial degradation for short (one week) and long term (three weeks) stability at different temperatures (Table 2). Concentrations obtained after six freeze thaw cycles resulted in residual vancomycin concentrations ranging from 95.6 to 101.9% compared to the initial vancomycin concentration determined on fresh plasma. Extract stability recovery for the three tested concentrations ranged from 86.0 to 114.0% after 24 and 48 h, indicating acceptable processed sample stability of our method for at least 48 h.

The average extraction recovery was 106.3% (4.8% CV) for vancomycin. The ME ranged from 9.1 to 118.2% (47.2% CV) and 34.2 to 85.9% (34.7% CV) for the 5 and 20 mg/L spiked vancomycin concentrations, respectively. When the response ratios (RR) (area vancomycin/area IS) were calculated for the different matrices, these ranged from 86.4 to 117.0% (9.4% CV) and 83.6–113.9% (10.9% CV) (5 and 20 mg/L vancomycin, respectively) as compared to the RR in pure solvent. The results for the different matrices are presented more in detail in Table 3. Matrix seven was the only matrix that showed ion enhancement. Compared to the other matrices that were taken on lithium heparin, this blood sample was taken on a serum separator tube with gel and clot activator, routinely not used for TDM of vancomycin. If the serum separator matrix is omitted from analysis, the CV on RR further improves to <8.5% for both levels. Of the 12 RR, 2 showed a ME (and hence accuracy) marginally worse than ±15% [20]. One was −16.4% at 20 mg/L and was taken on the serum separator tube, which is not routinely used; the other was +17.0% at 5 mg/L and was seen in a matrix of a patient that showed very substantial ion suppression. In our experience, such a remarkable suppression is encountered very rarely. During our method comparison, such low areas with respect to the calculated concentrations were not observed. The matrix was that of a patient receiving a multitude of medications (except vancomycin). The ME on the 20 mg/L experiment of this patient was 105% and well within limits, so a pipetting error cannot be excluded either. As small amounts of stock solution are spiked to the blank plasmas, relatively small pipetting errors can yield more substantial deviations in accuracy and imprecision.

The analysis of four plasma samples from patients that were on medication other than vancomycin proved the selectivity of the method; no peak signals in the retention time windows of vancomycin or vancomycin des-leucine were observed.

3.2. Method comparison

Bland-Altman, Passing & Bablok regression analysis and Pearson's correlation coefficients were used to compare the four immunoassays to our LC–MS/MS assay (Fig. 2). The regression indicated a proportional

Please cite this article as: Oyaert M, et al, Novel LC–MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact, Clin Chim Acta (2014), http://dx.doi.org/10.1016/j.cca.2014.12.012

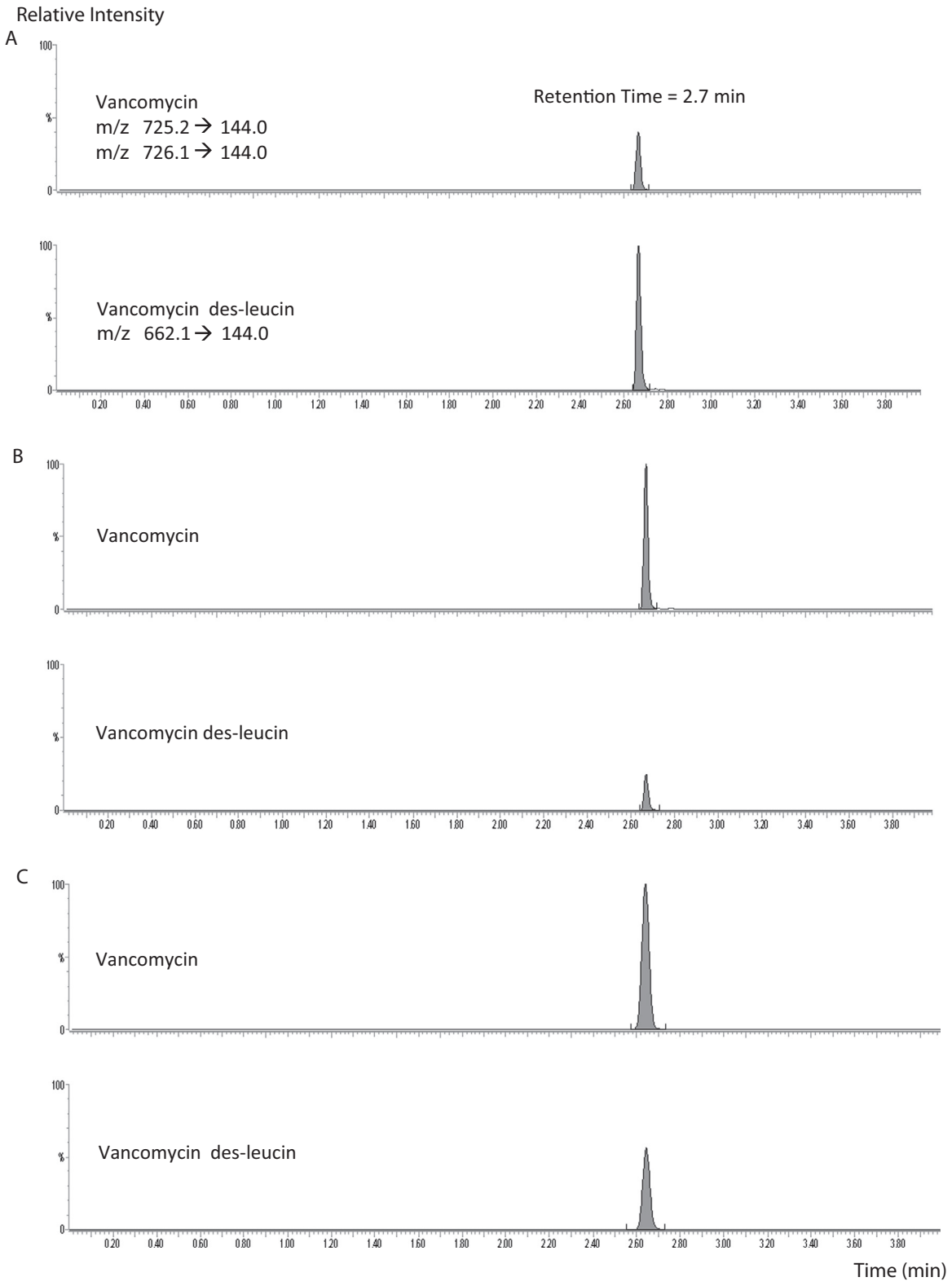


Fig. 1. A) Chromatogram of a low QC (3.0 mg/L vancomycin); B) chromatogram of a high QC (30.0 mg/L vancomycin); C) chromatogram of a patient sample (14.3 mg/L) vancomycin.

mean difference well within 10% for 3 out of 4 immunoassays. Only the Cobas 8000 assay showed a substantial proportional difference of +22% as compared to LC–MS/MS. Using Bland–Altman analysis, the highest mean difference was found for the Roche Cobas assay (19.3%; 95%CI: 16.5–22.0%) (Fig. 2). Inter-immunoassay Bland–Altman mean differences, Passing & Bablok regression analysis and Pearson's correlation coefficients are presented in Supplemental Table 1.

In the Bland–Altman analysis, the 95% limits of agreement (LOA) for the inter-immunoassay comparisons show ranges spanning 31.9%–48.4%, whereas the 95% LOAs for comparison of immunoassays with

Please cite this article as: Oyaert M, et al, Novel LC–MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact, Clin Chim Acta (2014), http://dx.doi.org/10.1016/j.cca.2014.12.012

Table 1
Imprecision of vancomycin LC–MS/MS analysis.

	Within-run imprecision			Total imprecision		
	Mean (mg/L)	SD (mg/L)	%CV	Mean (mg/L)	SD (mg/L)	%CV
Low QC	2.6	0.1	5.2	2.6	0.2	6.2
Medium QC	14.0	0.5	3.7	12.7	1.1	8.5
High QC	29.3	1.0	3.9	26.7	1.8	6.6
Ultra-high QC	75.8	2.0	2.8	70.3	5.5	7.9
Patient low	7.9	0.2	2.5	5.0	0.1	2.9
Patient medium	19.5	0.6	2.9	14.0	0.7	5.2
Patient high	48.9	1.7	3.5	27.8	0.7	2.6

LC–MS/MS give ranges spanning 47.6%–57%. This was not caused by a higher LC–MS/MS CV as the CVs on patient as well as QC samples (spiked plasma) for our method (2.6–8.5% on plasma) are not substantially different from the CVs reported in the method insert for the immunoassays (2.4–6.2% on iQC material). Residual ME in the LC–MS/MS method could be part of the explanation. The CV on the lithium heparin matrix RR, however, was <8.5% and for immunoassays no such analysis has been reported, so it remains difficult to judge the relative impact. Small calibration differences might also add to the larger LOA span.

Not surprisingly, clinical interpretation of the obtained concentrations with the different assays showed that the largest deviation with respect to the LC–MS/MS assay was found for the Cobas 8000 immunoassay (22.2% discordance). On the contrary, only 12.2%, 8.1% and 6.1% discordance was found between the Architect i2000SR, Vitros 5000 and Dimension Vista 15000 compared to LC–MS/MS, respectively. The distribution of the results is illustrated in Fig. 3.

4. Discussion

We developed and validated a novel LC–MS/MS method for quantification of total vancomycin in human plasma. All validation parameters were within the preset specifications. As our method was designed to be as user-friendly as possible, labor-intensive sample clean-ups and long runtimes to cope with matrix effects were avoided. The only sample pretreatment was a simple protein precipitation and the total analysis time was 5.0 min. The use of a hydrophilic interaction column (HILIC) ensured adequate retention of vancomycin. We also managed to avoid a protein precipitation with trifluoro/trichloro-acetic acid as described by previous groups [5,17,18]. In our experience, the use of strong acids is not beneficial to the life span of the chromatographic column, the tubings and the chromatographic system in general. Not surprisingly, we observed substantial matrix effects. Our results show undesirable accuracy and large variation on the vancomycin areas in different matrices. An impressive improvement was seen when the RR was used (from mean accuracy about 67% with a CV about 40% to a mean accuracy about 102% with a CV about 11%), indicating compensation by the IS. In contrast with other LC–MS/MS methods for vancomycin quantification [15–18], the structure of our IS was almost identical to that of the target analyte and ensured similar ionization and chromatographic behavior.

Commercially available automated immunoassays are widely used to quantify total vancomycin in serum or plasma. Although immunoassays allow easy and rapid analysis, a substantial between-method difference can be found. Next to standardization differences, plasma components (plasma proteins and salts) can vary between patients and therefore, interferences in binding of analytes to the antibody in immunoassays can differ, especially when the analyte is present in low concentrations [29,30]. In our experiment, 3 out of 4 immunoassays showed an acceptable difference with LC–MS/MS (<10%). Only the Cobas 8000 assay showed a significant proportional difference of >20.0%.

As Cobas 8000 measured invariably higher in the low, therapeutic as well as in the toxic range, there appears to be a standardization difference with the other assays. This difference causes a discordant clinical interpretation in more than 1 out of 5 samples. Given our results, and considering the clear-cut vancomycin therapeutic target concentration ranges, it is likely that clinical dosing decisions may depend on the assay that is used in an individual institution as suggested by Zhao et al. [8]. As it is difficult to trace the analytical details of the methods used for the determination of the therapeutic interval values [10], it remains unclear which of the tested assays gives values most similar to the originally used techniques. Some other studies explored between-assay differences. In this regard, a study by Azzazy et al. compared a FPIA method with a HPLC method for vancomycin measurement, and found satisfactory agreement between both methods [31]. Bijleveld et al. recently found only small differences in vancomycin

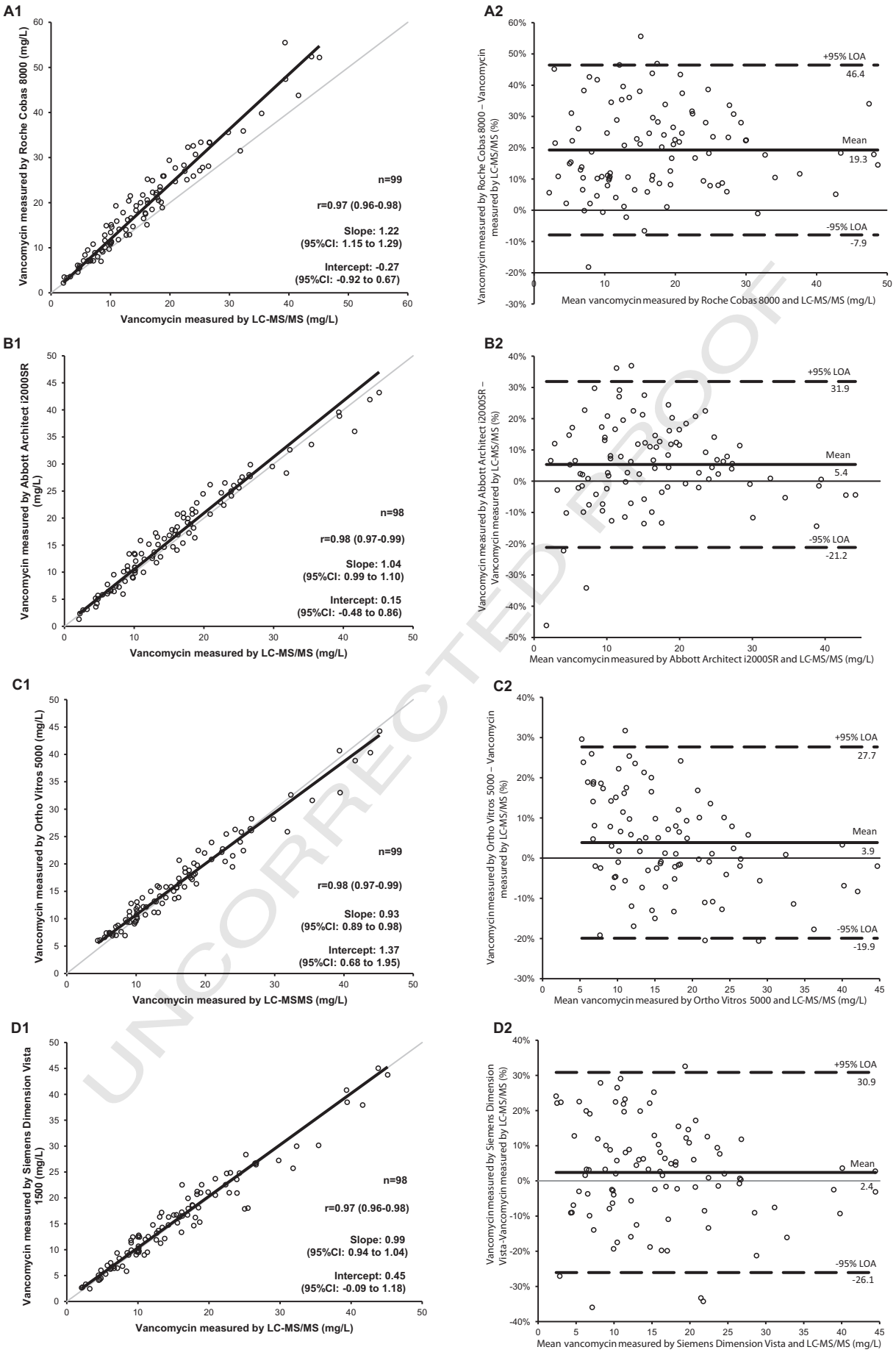
Table 3
Matrix effects (ME) and CVs for vancomycin LC–MS/MS analysis for 6 different matrices and water (matrix 1) for the A) 5 mg/L and B) 20 mg/L vancomycin experiments. ME for vancomycin and IS are presented as a percentage of the area obtained in water. ME for the response ratio (RR) was calculated as a percentage compared to the obtained RR in water. The ME expressed as a percentage of pure solvent area or RR was used as accuracy.

A	5 mg/L vancomycin experiment					
	Vancomycin		IS		RR	
	Area	ME	Area	ME	RR	ME
Matrix 1	1257	–	1313	–	0.963	–
Matrix 2	898	71.5%	860	65.5%	1.047	108.8%
Matrix 3	926	73.7%	901	68.6%	1.023	106.3%
Matrix 4	1149	91.4%	1231	93.8%	0.934	97.0%
Matrix 5	724	57.6%	707	53.9%	1.024	106.4%
Matrix 6	115	9.1%	102	7.8%	1.126	117.0%
Matrix 7	1486	118.2%	1936	147.5%	0.832	86.4%
Mean	**936**	**70.2%**	**1007**	**72.8%**	**0.992**	**103.6%**
CV	**47.2%**	**51.9%**	**56.6%**	**63.4%**	**9.4%**	**10.2%**
B	20 mg/L vancomycin experiment					
	Vancomycin		IS		RR	
	Area	ME	Area	ME	RR	ME
Matrix 1	6735	–	1768	–	3.830	–
Matrix 2	4485	66.6%	1046	59.2%	4.311	112.6%
Matrix 3	4623	68.6%	1052	59.5%	4.360	113.9%
Matrix 4	2793	41.5%	728	41.2%	3.800	99.2%
Matrix 5	5487	81.5%	1574	89.0%	3.487	91.1%
Matrix 6	2305	34.2%	571	32.3%	4.034	105.3%
Matrix 7	5782	85.9%	1803	102.0%	3.200	83.6%
Mean	**4601**	**63.0%**	**1220**	**63.9%**	**3.860**	**100.9%**
CV	**34.7%**	**33.3%**	**40.8%**	**42.2%**	**10.9%**	**11.9%**

Table 2
Stability of plasma samples. Values are expressed as a % recovery from the initial value.

Storage time	Concentration 1 (8.2 mg/L)			Concentration 2 (18.2 mg/L)			Concentration 3 (35.5 mg/L)		
	+20 °C	+8 °C	−20 °C	+20 °C	+8 °C	-20 °C	+20 °C	+8 °C	−20 °C
1 day	92.9	99.5	99.0	94.5	99.5	99.6	96.7	101.5	100.7
2 days	93.3	98.7	98.9	96.0	98.6	96.9	92.8	100.5	98.5
1 week	93.6	96.8	97.7	98.3	100.1	99.8	97.4	97.3	97.0
3 weeks	96.9	92.6	94.0	93.9	93.2	95.2	90.3	99.8	99.3

Please cite this article as: Oyaert M, et al, Novel LC–MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact, Clin Chim Acta (2014), http://dx.doi.org/10.1016/j.cca.2014.12.012



Please cite this article as: Oyaert M, et al, Novel LC–MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact, Clin Chim Acta (2014), http://dx.doi.org/10.1016/j.cca.2014.12.012

A

Discordance 22.2%		Roche Cobas 8000		
		<15 mg/L	15-25 mg/L	>25 mg/L
LC-MS/MS	<15 mg/L	43	10	0
	15-25 mg/L	0	20	12
	>25 mg/L	0	0	14

B

Discordance 12.2%		Abbott Architect i2000SR		
		<15 mg/L	15-25 mg/L	>25 mg/L
LC-MS/MS	<15 mg/L	45	7	0
	15-25 mg/L	2	27	3
	>25 mg/L	0	0	14

C

Discordance 8.1%		Ortho Vitros 5000		
		<15 mg/L	15-25 mg/L	>25 mg/L
LC-MS/MS	<15 mg/L	50	3	0
	15-25 mg/L	2	28	2
	>25 mg/L	0	1	13

D

Discordance 6.1%		Siemens Dimension Vista 1500		
		<15 mg/L	15-25 mg/L	>25 mg/L
LC-MS/MS	<15 mg/L	50	3	0
	15-25 mg/L	2	30	2
	>25 mg/L	0	1	13

Fig. 3. Clinical impact of interpretation of the obtained vancomycin concentrations (mg/L) with A) Roche Cobas 8000; B) Abbott Architect i2000SR; C) Ortho Vitros 5000 and D) Siemens Dimension Vista 1500 compared to LC–MS/MS. Results are presented as cross tables with the number of samples in each clinical interpretative category.

concentrations between their LC–MS/MS method and FPIA assay, with a slightly negative difference (−0.9% (95%CI: −6.8 to 5.1%)) [18]. More recently, Shipkova et al. compared 8 different immunoassays from three different manufacturers for TDM of vancomycin [6]. In their study, they found that all assays showed a proportional difference compared to the results obtained with the Cobas 8000 (12.0–19.0% lower). This is in accord with our results. Our study, however, adds one manufacturer and is able to give a comparison with reference technology.

We further examined the observed difference by performing a cross-analysis of standards. We analyzed the TDM Preciset (Roche standards) with our LC–MS/MS assay and our LC–MS/MS standards (spiked blank plasma) with the Cobas assay. This analysis, however, didn't produce conclusive evidence. The TDM Preciset standards gave results within ±15% of the indicated values and the LC–MS/MS standards gave results within ±16% of the theoretical values. It appears that other factors are the main cause of the observed difference. Roche standards analyzed with Roche reagent on Cobas 8000 c702 might suffer from a differential ME as compared to true vancomycin patient samples. Also, several structurally related impurities can be present in vancomycin preparations. Diana et al. found 15 different impurities, together composing 16.6% of the sample [7]. The variation of these impurities with different lots of vancomycin or the impact on different immunoassays has never been examined into detail. As most of these metabolites have substantially different molecular masses or show different polarities, LC–MS/MS methods are least likely to suffer from interference by these compounds. The Roche method might suffer more from interference (or cross-reactivity) by these components or other substances present in samples from patients receiving vancomycin. A limitation of our study, however, is that lot-to-lot variability of the different assays was not included. Other lots of calibrators and reagents might give rise to slightly different results.

In conclusion, we developed an applicable LC–MS/MS method for vancomycin measurement in human plasma. Correlation of our LC–MS/MS method with four immunoassays showed substantial differences with the Cobas 8000 assay, causing discordant clinical interpretation in more than 1 out of 5 samples. Therefore the transferability of vancomycin results between laboratories has to be interpreted with caution.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cca.2014.12.012.

5. Uncited reference

[22]

Acknowledgments

We kindly thank Pharm. Clin. Biol. E. Jacobs of the H. Hart Hospital Lier for assistance with Ortho Vitros 5000. Karel Allegaert, Pieter Vermeersch (fundamental clinical investigatorship 1800214N and 1842013N, respectively) and Steven Pauwels (Clinical fellowship 1700314N) are supported by the Fund for Scientific Research, Flanders. Isabel Spriet has clinical research funding from the University Hospitals Leuven.

References

[1] Finch RG, Eliopoulos GM. Safety and efficacy of glycopeptide antibiotics. J Antimicrob Chemother 2005;55(Suppl. 2):ii5–ii13.
[2] Steinkraus G, White R, Friedrich L. Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-susceptible clinical methicillin-resistant *S. aureus* (MRSA blood isolates from 2001–05). J Antimicrob Chemother 2007;60:788–94.
[3] Gould IM. Clinical relevance of increasing glycopeptide MICs against *Staphylococcus aureus*. Int J Antimicrob Agents 2008;31(Suppl. 2):1–9.
[4] Wilson JF, Davis AC, Tobin CM. Evaluation of commercial assays for vancomycin and aminoglycosides in serum: a comparison of accuracy and precision based on external quality assessment. J Antimicrob Chemother 2003;52:78–82.
[5] König K, Kobold U, Fink G, et al. Quantification of vancomycin in human serum by LC–MS/MS. Clin Chem Lab Med 2014;51:1761–9.
[6] Shipkova M, Petrova D, Rosler A, et al. Comparability and imprecision of 8 frequently used commercially available immunoassays for therapeutic drug monitoring. Ther Drug Monit 2014;36:433–41.
[7] Diana J, Visky D, Hoogmartens J, Van Schepdael A, Adams E. Investigation of vancomycin and related substances by liquid chromatography/ion trap mass spectrometry. Rapid Commun Mass Spectrom 2006;20:685–93.
[8] Zhao W, Kaguelidou F, Biran V, et al. External evaluation of population pharmacokinetic models of vancomycin in neonates: the transferability of published models to different clinical settings. Br J Pharmacol 2012;75:1068–80.
[9] Zhao W, Jacqz-Aigrain E. The importance of knowing how vancomycin is measured when interpreting its pharmacokinetic results. Ther Drug Monit 2013;35:416.
[10] Kullar R, Davis SL, Levine DP, Rybak MJ. Impact of vancomycin exposure on outcomes in patients with methicillin-resistant *Staphylococcus aureus* bacteremia: support for consensus guidelines suggest targets. Clin Infect Dis 2011;52:975–81.
[11] Rybak M, Momaestro B, Rotschafer JC, et al. Therapeutic drug monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. Am J Health Syst Pharm 2009;66:82–98.
[12] Mouton JW, Theuretzbacher U, Craig WA, Tulkens PM, Derendorf H, Cars O. Tissue concentrations: do we ever learn? J Antimicrob Chemother 2008;61:235–7.
[13] Lamer C, de Beco V, Soler P, et al. Analysis of vancomycin entry into pulmonary lining fluid by bronchoalveolar lavage in critically ill patients. Antimicrob Agents Chemother 1993;37:281–6.

Fig. 2. Method comparison of the vancomycin assays with Passing Bablok (1) and Bland–Altman (2) analysis of LC–MS/MS versus Abbott Architect i2000SR (A), Roche Cobas 8000 (B), Ortho Vitros 5000 (C), Siemens Dimension Vista 1500 (D).

Please cite this article as: Oyaert M, et al, Novel LC–MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact, Clin Chim Acta (2014), http://dx.doi.org/10.1016/j.cca.2014.12.012

[14] Rybak MJ. The pharmacokinetic and pharmacodynamic properties of vancomycin. Clin Infect Dis 2006;42(Suppl.1):S35–9.
[15] Cass RT, Villa JS, Karr DE, Schmidt Jr DE. Rapid bioanalysis of vancomycin in serum and urine by high-performance liquid chromatography tandem mass spectrometry using on-line sample extraction and parallel analytical columns. Rapid Commun Mass Spectrom 2001;15:406–12.
[16] Zhang T, Watson DG, Azike C, et al. Determination of vancomycin in serum by liquid chromatography–high resolution full scan mass spectrometry. J Chromatogr B 2007; 857:352–6.
[17] Shibata N, Ishida M, Prasad YV, Gao W, Yoshikawa Y, Takada K. Highly sensitive quantification of vancomycin in plasma samples using liquid chromatography–tandem mass spectrometry and oral bioavailability in rats. J Chromatogr B 2003;787: 211–8.
[18] Bijleveld Y, de Haan T, Toersche J, et al. A simple quantitative method analyzing amikacin, gentamicin, and vancomycin levels in human newborn plasma using ion-pair liquid chromatography/tandem mass spectrometry and its applicability to a clinical study. J Chromatogr B 2014;951:110–8.
[19] CLSI. Evaluation of precision performance of quantitative measurement methods; approved guideline—second edition. CLSI Document EP05-A2. Wayne, PA: Clinical Laboratory Standards Institute; 2004.
[20] European Medicines Agency, guideline on bioanalytical method validation. 21 July 2011; 2011.
[21] CLSI. Mass spectrometry in the clinical laboratory: general principles and guidance; approved guideline. CLSI Document C50-A. Wayne, PA: Clinical Laboratory Standards Institute; 2007.
[22] Kees MG, Wicha SG, Seefeld A, Kees F, Kloft C. Unbound fraction of vancomycin in intensive care unit patients. J Clin Pharmacol 2014;54:318–23.
[23] Abbott Architect iVancomycin G3, package insert; 11-2012.
[24] Roche Vancomycin VANC2, package insert 08-2013,V2; 2013.
[25] Ortho Vitros 5000 Vancomycin VANC, package insert 05-2012, V4; 2012.
[26] Siemens Dimension Vista Vancomycin VANC, package insert; 11-2012.
[27] Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–10.
[28] Passing H, Bablok W. A new biometrical procedure for testing equality of measurements from two different analytical methods. J Clin Chem Clin Biochem 1983;21: 709–20.
[29] Ezan A, Emmanuel A, Valente D, Grognet JM. Effect of variability of plasma interferences on the accuracy of drug immunoassays. Ther Drug Monit 1997;19:212–8.
[30] LeGatt DF, Blakney GB, Higgins TN, et al. The effect of paraproteins and rheumatoid factor on four commercial immunoassays for vancomycin: implications for laboratorians and other health care professionals. Ther Drug Monit 2012;34:306–11.
[31] Azzazy HM, Chou PP, Tsushima JH, et al. Abbott AxSYM vancomycin II assay: multicenter evaluation and interference studies. Ther Drug Monit 1998;20:202–8.

Please cite this article as: Oyaert M, et al, Novel LC–MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact, Clin Chim Acta (2014), http://dx.doi.org/10.1016/j.cca.2014.12.012